

Changes in the Chemical Speciation of Arsenic Following Ingestion by Man

by Eric A. Crecelius*

The concentrations of four chemical species of arsenic in urine were observed with time, after ingestion of three different chemical species of arsenic. The arsenic-rich substances ingested, included arsenite-rich wine, arsenate-rich drinking water, and crab meat which contained organo-arsenic compounds. After ingestion of arsenite-rich wine, approximately 10% of the arsenic was excreted as arsenite, but the majority of the arsenic was methylated to methylarsonic acid and dimethylarsinic acid and excreted. After ingestion of arsenate-rich water, elevated levels of both arsenate and dimethylarsinic acid were observed. When crab meat was ingested, none of these four arsenic species were observed at elevated levels until the urine was heated in 2N NaOH. After the hot base digestion, high levels of dimethylarsinic acid were detected in these samples. The apparent biological half-lives were on the order of 10 hr for inorganic arsenic and 30 hr for the methylated arsenic forms.

Introduction

Of the chemical forms of arsenic to which man is normally exposed, trivalent arsenicals are the more toxic. Little information is available on the changes in the chemical forms of arsenic that occur within the body. However, reviews and summaries on the biochemistry of arsenic in man (1-7) indicate that (1) arsenic which enters the blood stream is excreted mainly in the urine; (2) arsenic has a biological half-life of 30-60 hr; and (3) arsenic is excreted in urine in several forms, including arsenite (As^{+3}), arsenate (As^{+5}), methylarsonic acid (MAA), dimethylarsinic acid (DMAA), and other organically bound arsenic compounds. Other information on the behavior of arsenic in the human body that is either contradictory or unsupported, include such suggestions as: As^{+3} accumulates in the body (5); As^{+3} is oxidized to As^{+5} in the body and then excreted (3); and As^{+3} and/or As^{+5} are methylated in the body (7, 8).

Because As^{+3} is much more toxic than As^{+5} , MAA, and DMAA, processes that convert As^{+3} to other forms may be part of the body's normal protective response to detoxification. The purpose of this study was to determine in a semiquantitative manner the chemical species and excretion rates in

urine following ingestion of known arsenic species. The results were expected to provide insight into the possible arsenic reactions and their rate within the human body.

Methods and Materials

The analytical technique used to determine the urinary arsenic species was similar to that described by Braman and co-workers (8, 9), but with modifications described by Crecelius (10). The species of arsenic were selectively reduced to volatile arsenic compounds and then detected using an emission spectrometer equipped with a helium plasma excitation source. In the procedure, four species: As^{+3} , As^{+5} , MAA, and DMAA, are selectively reduced to the gaseous compounds arsine, methylarsine, and dimethylarsine, by controlled pH and by sodium borohydride (NaBH_4) as a reducing agent. The other organically bound arsenic, defined here as arsenic compounds not reduced to a gas by NaBH_4 , was analyzed after performing either a hot 2N NaOH digestion, or a hot acid digestion of the urine with sulfuric, nitric and perchloric acids. After the NaOH digestion, which does not decompose the MAA or DMAA, the samples can be analyzed for inorganic and methylated arsenic. However, after the hot acid digestion, all the arsenic was inorganic and analyzed for As^{+5} by using the same reduction procedure.

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The subject studied in these arsenic ingestion experiments was a 30 yr-old male with a body weight of 70 kg. The three materials ingested were domestic table wine, well water, and canned Dungeness crab (*Cancer magister*) meat. The diet of the subject was controlled to minimize the intake of arsenic from any food and drink before and during the experiments.

Wine was chosen because it is a common beverage which often contains relatively high concentrations as As^{+3} (100–500 $\mu\text{g}/\text{l.}$, unpublished data). The predominant arsenic species in wine is the As^{+3} with lesser amounts of As^{+5} . No MAA or DMAA was detected in the wine. The arsenic-rich well-water that was used for the experiments, had been used for domestic water supply. The chemical form of arsenic in the well water was As^{+5} .

The chemical form of the majority of the arsenic in the crab was not identified. When the cooked crab tissue was homogenized in distilled water, only a few percent of the arsenic could be converted to arsines; however, after the tissue was digested overnight in 2N NaOH at 80°C, approximately 90% of the total arsenic was present as DMAA. Apparently, the arsenic in several marine animals, including crab, shrimp, and fish, is present as a stable organic arsenic compound that can be broken down into DMAA (unpublished data).

Results and Discussion

The concentrations of As^{+3} , As^{+5} , MAA and DMAA in urine before and after the ingestion of As^{+3} -rich wine are shown in Figure 1. The amounts of each arsenic species excreted are shown in Figure 2. In the experiment, 450 ml of wine, containing 50 μg As^{+3} and 13 μg As^{+5} was ingested. Before ingestion, the urinary arsenic level was near the normal level of 15 ppb. Within 5–10 hr after ingestion, the urinary levels of As^{+3} , As^{+5} , MAA, and DMAA had each increased by about a factor of five. The levels of As^{+3} and As^{+5} rapidly decreased and were near normal 20 hr after ingestion. However, the MAA and DMAA levels remained elevated, reaching the maximum levels about 40 hr after ingestion, then gradually approached normal levels 85 hr after ingestion. The relatively low concentrations of DMAA and MAA measured at about 13 hr (Fig. 1) was caused by dilution of the urinary arsenic levels because the intake of liquids by the subject was not constant. When the amount, instead of the concentration, of arsenic excreted with time is plotted (Fig. 2), dilution effects are eliminated, but the data are then biased by the length of time between urine samples. Note that during the time interval between 38 and 85 hr only

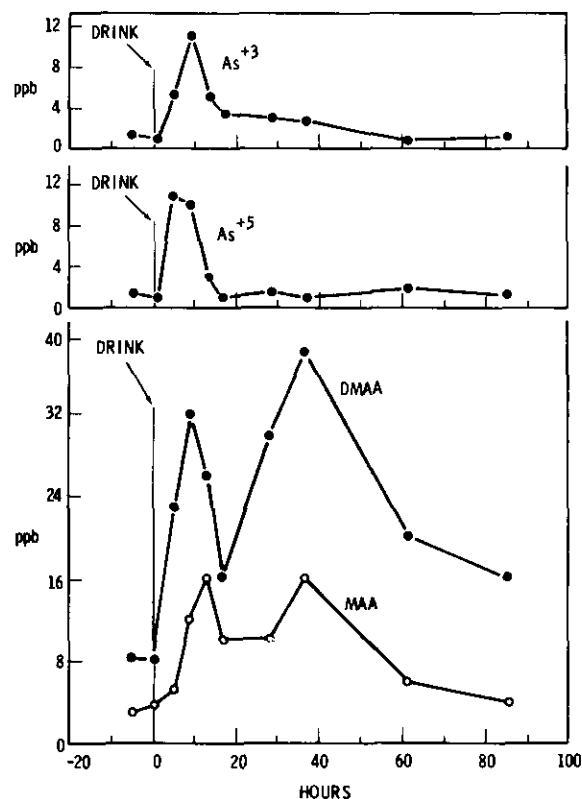


FIGURE 1. Concentrations of arsenic compounds in urine with time after ingestion of wine containing 50 μg As^{+3} and 13 μg As^{+5} . The concentrations of methylarsonic acid (MAA) and dimethylarsinic acid (DMAA) are given in ppb as arsenic.

one urine sample was analyzed, although several were collected.

The arsenite-rich wine ingestion experiment was repeated a month later with a different wine containing similar levels of arsenic species. The same general variations in urinary arsenic species with time were observed.

A budget was calculated for the first experiment (Fig. 2) to compare the amount of arsenic ingested with the amount excreted. A urinary arsenic level of 15 ppb was considered a normal background level. Of the 63 μg of arsenic ingested, approximately 80% was excreted in the urine within 61 hr. The major species of arsenic excreted was DMAA which accounted for about 50% of the 63 μg of arsenic ingested. Arsenite and As^{+5} each accounted for 8% and MAA for 14%.

Several urine samples were analyzed for organically bound arsenic (non-reducible arsenic) by first digesting with hot acids and then analyzing for As^{+5} . This procedure indicated that organically-bound arsenic accounted for an insignificant amount of the arsenic in these samples.

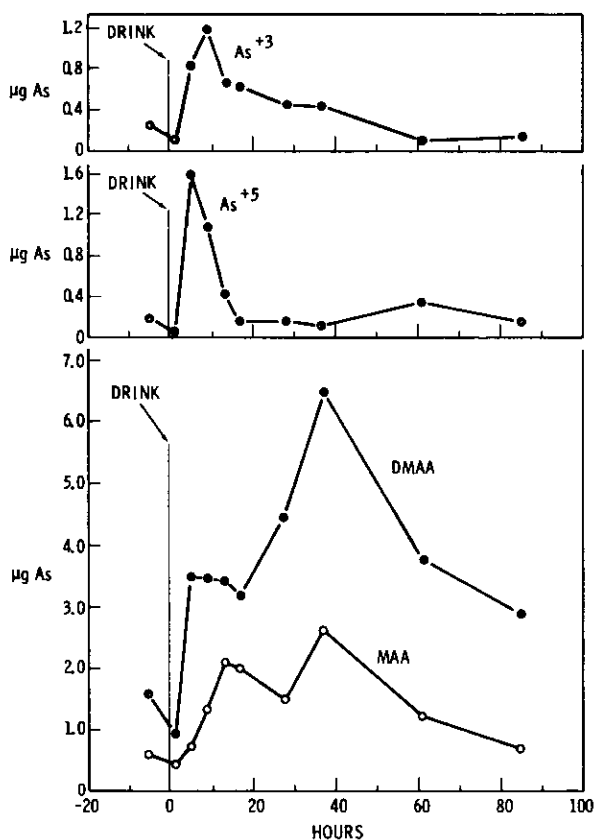


FIGURE 2. Amount of arsenic compounds in urine with time after ingestion of wine containing 50 μg As^{+3} and 13 μg As^{+5} . The amount of methylarsonic (MAA) acid and dimethylarsinic (DMAA) acid are given in μg as arsenic.

The concentration of four arsenic species in urine after the ingestion of As^{+5} -rich well water is shown in Figure 3. In the experiment, 200 μg of As^{+5} was ingested. After ingestion, the As^{+3} concentration in urine was near normal levels of 1 to 2 ppb. The As^{+5} concentration in urine showed a marked increase within the first 10 hr after ingestion, indicating some of the As^{+5} ingested was rapidly excreted. The concentration of DMAA increased slightly between 10 and 70 hr after ingestion. A mass balance for the As^{+5} -rich well water ingestion experiment could account for less than 50% of the arsenic ingested being excreted in the urine during the 70 hr following ingestion.

The concentrations of arsenic species in urine after ingestion of canned Dungeness crab are shown in Figure 4. In this experiment, 340 g (wet weight) of crab meat containing approximately 2000 μg of an unidentified organo-arsenic compound was ingested. The four arsenic species showed no significant changes during the 60 hr following ingestion (upper graph, Fig. 4). However, when urine sam-

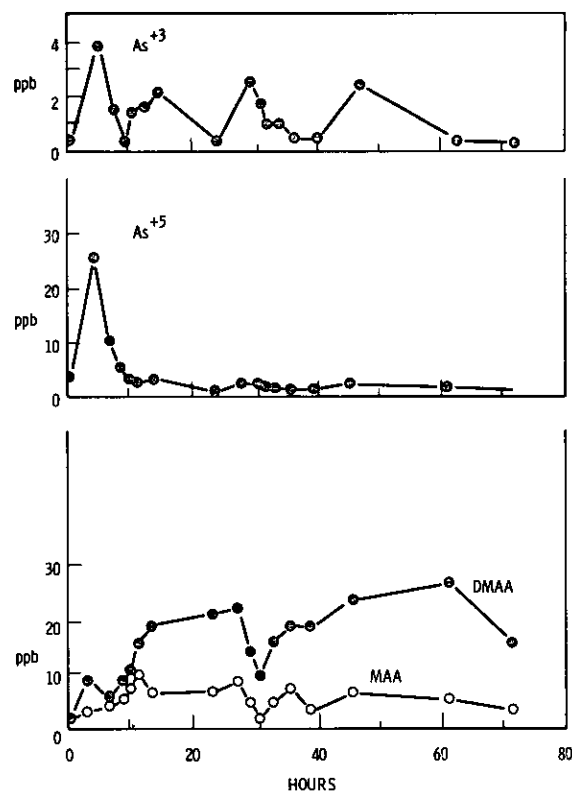


FIGURE 3. Concentration of arsenic compounds in urine with time after ingestion of well-water containing 200 μg As^{+5} . The concentration of MAA and DMAA are given in ppb as arsenic.

ples were digested with hot 2N NaOH, high concentrations of DMAA were observed (lower graph, Fig. 4). These data indicate that the organo-arsenic compound in crab is excreted from the body in the urine within 1–2 days after ingestion, and the arsenic is not broken down to inorganic arsenic in the body. Chapman (11) has noted that after ingestion of arsenic-rich tissue from lobster, the majority of the arsenic in urine is organically bound and not reducible until rigorously digested.

The results of these experiments provide information on the fate of As^{+3} , As^{+5} and an organic arsenic compound ingested by man. When As^{+3} is ingested, a small portion is excreted as As^{+3} , but the majority of As^{+3} is methylated in the body and excreted as DMAA and MAA. There appear to be two different processes with different rates removing the ingested As^{+3} . The first process is the excretion of As^{+3} which presumably was assimilated into the blood then removed by the kidneys. The rapid excretion process began 5 hr after ingestion, had diminished 20 hr after ingestion, and was not evident 60 hr after ingestion. The second process of As^{+3} removal is its methylation to MAA and

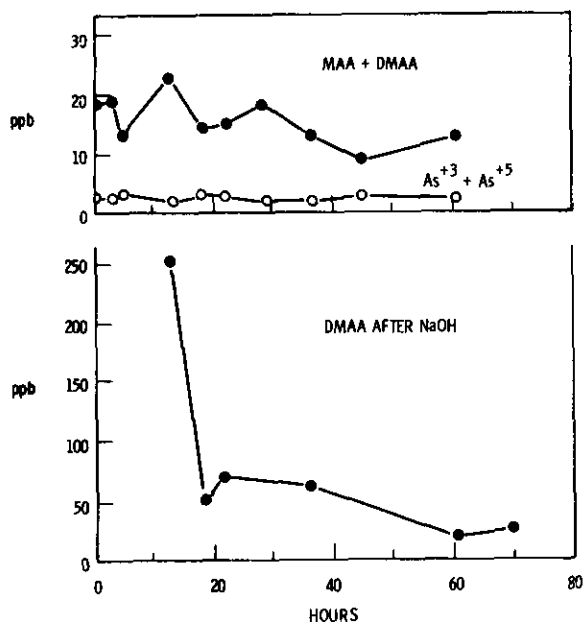


FIGURE 4. The upper graph shows the concentration of arsenic compounds in urine with time after ingestion of crab meat containing 2000 μg of an organo-arsenic compound. The lower graph shows the concentration of DMAA in the same urine samples after NaOH digestion. The concentration of MAA and DMAA are given in ppb as arsenic.

DMAA. The excretion of methylated arsenic began within five hr after ingestion but reached maximum levels much later than As^{+3} . The substantially different excretion pattern for methylated arsenic may be due to As^{+3} reacting or binding with active sites in the body and then gradually being methylated and excreted. Most of the arsenic ingested was excreted within 85 hr and the apparent biological half-life was on the order of 30 hr, similar to that reported by others (4).

The fate of As^{+5} in the body is difficult to assess from these data because a mass balance of the As^{+5} ingested could account for less than 50%. However,

urinary As^{+5} levels after ingestion suggest some As^{+5} is rapidly excreted from the body without any changes in chemical form. Part of the As^{+5} was methylated and excreted within several days.

When organo-arsenic from marine animals is ingested, the arsenic compound appears to be quickly excreted in the urine without changes in the chemical form.

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